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Effects of grassland management on plant nitrogen use efficiency (NUE): evidence from a long-term experiment

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Abstract

Grassland management intensification can greatly influence nitrogen (N) dynamics between aboveground and belowground compartments mainly because of the large amount of available N forms, which are repeatedly added to soils. A better understanding of how chronic fertilisation might affect N use efficiency (NUE) in plants can contribute to reducing N losses from soils and improve the sustainability of managed grasslands. Here we address how NUE might be affected by (1) the addition of key nutrients (e.g. N, P, K, Mg) in different combinations, (2) grazing by rabbits, and (3) liming (i.e. $CaCO_3$ applications) in a 22-year-old permanent grassland experiment established in Berkshire, UK, in 1991. We first calculate seven different NUE indexes, which are known to respond either to changes in soil N availability (i.e. endogenous N inputs from soil N mineralization processes) or to exogenous N inputs (i.e. synthetic N fertiliser). We found that plant NUE calculated as plant biomass produced per unit of N acquired significantly decreased under the chronic addition of multiple nutrients (NPKMg) and was even lower under N-only applications. Most NUE indexes significantly decreased under grazing but greatly increased under liming applications. We found evidence that NUE indexes, which accounted for endogenous N sources decreased at increased rates of soil N mineralization. Finally, we found no significant relationships between any of the NUE indexes and estimates of soil N losses (Mg N ha^{-1}) or N retention in soils (i.e. units of soil N retained per unit of N added) calculated from changes in net soil N budget over 22 years. Our study carried out on relatively acidic sandy soils suggests how liming applications in combination with low levels of multi-nutrient additions (NPKMg) can significantly improve plant biomass production per unit of N added thus contributing to enhance the sustainability of managed grassland ecosystems.

Keywords: grassland intensification, grazing, liming, nutrient fertilisation, plant yields

Introduction

Human-managed grassland ecosystems are often artificially 'improved' to increase their primary productivity (i.e. grass yields). Grassland improvement has been mainly achieved through regular nutrient fertilisation (e.g. Nitrogen (N), Phosphorus (P) additions to soils), liming (i.e. the application of Calcium and Magnesium-rich materials to grassland soils) and livestock grazing (Conant et al. 2001; Blüthgen et al. 2012; Heyburn et al. 2017). A common feature of these management practices is that either directly or indirectly they greatly contribute to increase soil N availability, which is a key factor sustaining plant productivity. However, increased soil N inputs may be associated with a strong decrease in plant species diversity (Fornara & Tilman 2012) and with negative N fertilisation effects on important ecosystem services, such as: (i) increased N mineralisation (Zhang et al. 2012) and greater potential for N loss, and (ii) a decline in soil fungal abundance (de Vries et al. 2006; Weber et al. 2013). To improve the sustainability of managed grassland ecosystems we need to better understand how plants might uptake and use N under the effects of multiple practices including N fertilisation, grazing and liming.

In this study we use a long-term ecological experiment to address how Nitrogen Use Efficiency (NUE) might respond to multiple grassland management practices. We assume that variations in NUE result from differences in nutrient retention and acquisition by plants (Hiremath & Ewel, 2001) and we use a set of seven indices to specifically assess how applied N (i.e. exogenous N additions) and soil N availability (i.e. endogenous N) are utilised to produce biomass (Pastor & Bridgham 1999; Dobermann 2005).

The basic tenet is that plant communities growing on artificially N-fertilised soils are more productive but have significantly lower NUE when compared to less productive communities growing on N-poor soils, which have higher NUE (Pastor & Bridgham 1999; Yuan et al. 2006). NUE theory suggests that plant responses to changes in soil N availability

74 can be either (1) monotonic where plant NUE increases as soil N availability decreases (Yuan
75 et al. 2006; Keuter et al. 2013), or (2) unimodal where plant NUE initially increases with
76 increasing soil N availability but eventually decreases when soil N availability reaches
77 critical levels (Pastor & Bridgham 1999). In both models (monotonic and unimodal) NUE
78 eventually decreases in response to chronic N fertilisation, therefore we expect to find similar
79 NUE responses in managed grasslands, which received repeated N additions for many years.
80 Grazing can also affect NUE because mammalian herbivores tend to create and
81 maintain a grassland community, which has higher relative yields compared to ungrazed
82 pastures and also improved digestibility of forages (McNaughton 1984; Kleinebecker et al.
83 2011). A first mechanism by which mammalian grazers can influence plant N uptake and
84 NUE is through the addition of labile N forms to soils from animal faeces and urine (van der
85 Wal et al. 2004). A second mechanism is associated with increased plant tolerance to
86 herbivore damage, which may include higher plant compensatory growth, increased
87 photosynthetic activity, phenological delays and relocation of resources to storage reserves
88 (Tiffin 2000), all of which influence NUE. Measuring NUE when plant biomass is removed
89 by grazers remains challenging mainly because plant biomass:N content ratios vary
90 seasonally according to changes in the frequency and intensity of herbivory and changes in
91 plant growth (see Kleinebecker et al. 2011).
92 The addition of liming materials to soils (e.g. $CaCO_3$) can increase NUE by altering
93 the availability of inorganic forms of soil N (e.g. NO_3) for plant uptake as found in an acidic
94 upland grassland (Ignacio Rangel-Castro et al. 2004). Liming can also indirectly increase
95 soils' biological capacity for N fixation and nitrification as found in an acidic subnatric
96 yellow sodosol (Wakelin et al. 2009), for example, by favouring the establishment and
97 persistence of legume species within grassland plant communities. Finally, liming may

influence NUE because of its positive effects on the size of mineral-associated soil organic matter pools, which can retain more N (Briedis et al. 2012).

Despite experimental evidence that N fertilisation, grazing and liming can significantly affect NUE, very few studies have so far addressed the long-term effects of these three practices on NUE. Greater NUE by plants could help reduce overall N fertilisation rates and decrease N leaching from soils (Dobermann 2005). There is also scope to improve food production per unit of N added to soils, this is because extensively managed grasslands with good NUE can result in good forage quality (Klingler et al. 2018). However, it could also be that changes in soil N content lead to a reduction in forage quality (Wilkins, Allen & Mytton, 2000).

Here we specifically address how 22 years of different nutrient fertilisation, grazing (by rabbits) and liming treatments (1) have influenced plant NUE, and (2) whether changes in NUE are potentially related to changes in soil processes such as soil N mineralization or the ability of grassland soils to retain or lose N.

In order to produce estimates of NUE, which are comparable with previous estimates, we applied seven different calculation methods, which have been used and reported in multiple literature studies (Berendse & Aerts 1987; Pastor & Bridgham 1999; Dobermann, 2005; Yuan et al. 2006). We specifically compare two sets of NUE indexes: (a) the first set measures productivity in response to N acquisition and N availability within the soil; (b) the second set of indexes (derived from Dobermann 2005), measures productivity in response to applied N fertiliser (see details in the Methods section).

To our knowledge, no studies have so far simultaneously addressed potential long-term effects of multiple management practices (i.e. nutrient fertilisation, liming and grazing) on plant NUE. Here we use a well-replicated grassland experiment established in 1992 at Silwood Park, Berkshire, UK to test the following hypotheses:

H_1 NUE will be lower under chronic N fertilisation (either when N is applied alone or in combination with other nutrients) and will be higher in unfertilised grassland soils;

H_2 Chronic grazing will be associated with lower plant NUE;

H_3 Long-term liming applications will be associated with greater NUE;

H_4 NUE will be negatively related to the availability of inorganic soil N forms (i.e. high NUE associated with low rates of soil N mineralization);

H_5 N losses from soils will be lower where plant NUE is higher.

Materials and methods

Experimental Design & Treatments

Our study was carried out in Nash's Field, a mesotrophic grassland (*Lolio-Cynosuretum cristati* grassland, *Anthoxanthum odoratum* sub-community; Rodwell 1992), with acid, sandy soils established at Silwood Park, Berkshire. The experiment started in 1991 to test for the effects of various treatments on plant community structure. The experiment is laid out in a split-plot design (3 factor factorial) with four larger plots (22 x 44 m), hereafter blocks, each split to contain two grazing treatments (± grazing), within these there are two pH treatments (± lime), which are in turn sub-divided to contain five (2 x 2 m) plots receiving different nutrient treatments. Thus, the split plot nested design includes 4 blocks x 2 grazing treatments x 2 liming treatments x 5 nutrient treatments. In the statistical model, the four large blocks were included as random effects while the treatment factors were considered as fixed effects. Lime was applied at 5 tonnes of $CaCO_3$ ha^{-1} every few years to maintain a soil pH close to 7 and mineral nutrients were applied annually at the following rates: ammonium nitrate (N) 100 kg ha^{-1}, triple superphosphate (P) 35 kg ha^{-1}, muriate of potash (K) 225 kg ha^{-1} and Epsom salts (Mg) 11 kg ha^{-1}. The combination of nutrient treatments was as follows: (i) N-only, (ii) P-only, (iii) N, P and Mg together, (iv) simultaneous addition of all nutrients (N, P, K and

148 Mg) and (v) unfertilized-control. Exclusion fences, that are rabbit proof, are present at this
149 ongoing experiment to test for the effects of grazing, primarily by rabbits (*Oryctolagus*
150 *cuniculus* L.). Occasional grazers include muntjuc (*Muntiacus reevesi* Ogilby) and roe
151 (*Capreolus capreolus*) deer. Within the grazing exclusion plots, biomass has been cut with a
152 sickle bar mower (and removed from plots) in August each year since the summer of 1991.
153 Rabbits are able to move across unfenced plots, which receive nutrient and liming treatments.
154 The soils of Nash's Field are characterized by low P status and have a mean total P value of
155 5.9 mg kg^{-1}.

156 Plant species composition of Nash's Field is dominated by the following grass
157 species: Common Bent (*Agrostis capillaris*), False Oat Grass (*Arrhenatherum capillaris*),
158 Red Fescue (*Festuca* rubra), Creeping Soft Grass (*Holcus* mollis) and Yorkshire Fog (*Holcus*
159 *lanatus*) (Allan & Crawley 2011). Grasses comprise 74% of the standing biomass across all
160 treatments, while forbs contribute 22.2% and legumes 1.1% on average. Abundant forb
161 species include Goose Grass (*Galium aparine*), Sorrel (*Rumex acetosa*), Germander
162 Speedwell (*Veronica chamaedrys*) and Ragwort (*Senecio jacobaea*). Legume species include
163 Lesser Trefoil (*Trifolium dubium*), White Clover (*Trifolium repens*) and Common Vetch
164 (*Vicia sativa*).

165

166 *Soil sampling and analysis*

167 Five soil cores were collected between 6th and 7th of May 2014 to a depth of 20 cm
168 from each of the 80 experimental plots and homogenised to form one sample. From these
169 samples, subsamples were taken to enable the following analysis: soil total C, N and P. Soil
170 samples were dried at 40°C for 5 days, passed through a 2 mm sieve to remove roots and
171 stones and ground in a ball mill. Care was taken to remove all visible roots with tweezers.
172 Samples were analysed for total C, N and P concentration by combustion and gas

chromatography using a COSTECH Analytical ECA 4010 instrument at the Ecosystems Analysis Laboratory, University of Nebraska, Lincoln, USA. Total P was determined with the sodium hydroxide (NaOH) fusion method described by Smith and Bain (1982).

Soil bulk density (BD) was measured from samples collected on the 7th May 2014 for each of the 80 plots with a soil corer (20 cm deep and 8 cm diameter) and calculated by dividing the mass of oven-dried soil by total soil volume. Soil samples were dried at 50 ˚C until a constant weight. Percentage soil moisture was calculated by dividing the mass of oven dry soil by the mass of fresh soil and multiplying this by 100 (Blake & Hartge 1986).

A further 80 soil samples were collected on the 18th of June 2014 at a depth of 20 cm to measure N mineralization rates. Samples were sieved through a 2 mm mesh and 20 g of soil was placed in each of 160 individual urine pots (i.e. two pots per sample). A first sub-sample of 80 vials was used for the first extraction where 50 ml of 1M KCl solution (149.1 g KCl to 1L of DI water) was added to each vial before soils were shaken for 30 minutes to homogenise the solution. These soils were then settled overnight at 4 °C. After, 5 mL of the clear supernatant that had separated from the now settled soil, was aliquoted into 80 x 8 mL bijou vials and frozen. The remaining 80 sub-sample vials were incubated in the dark at a constant moisture and temperature (22 °C) for 30 days. Sufficient water was added to each sample to reach the assumed field moisture capacity of 9% and again after 2 weeks during the 30-day laboratory incubation if necessary to keep moisture constant. Thereafter, 50 ml of 1M KCl solution was added to the samples which were then incubated overnight. From each of the 30-day exposure samples, 5 mL of the settled clear supernatant was pipetted into individual 8 mL bijou vials and frozen. All samples were analysed for NH_4^+-N and NO_3^--N with a Bran-Luebbe AA3 auto analyser. Net N mineralization was calculated by subtracting initial extractable nitrate and ammonium concentrations from the final concentrations measured after soils were incubated for 30 days (Robertson et al. 1999).

198 *Plant aboveground biomass, plant percent cover and diversity*

199 We measured plant aboveground biomass and analysed available data on plant
200 community composition. Aboveground plant biomass was harvested inside a 25 × 25 cm
201 quadrat placed in the centre of each 2 x 2 m plot between 10th and 19th June 2014. Samples
202 were dried for 4 days at 65°C and weighed, ground and placed in 1.0 mL Eppendorf tubes
203 and analysed for total C and N by combustion and gas chromatography using a COSTECH
204 Analytical ECA 4010 instrument. Plant percent cover including percent of each of three
205 functional groups (C_3-grasses, legumes and forbs) was estimated and agreed by two
206 independent investigators within each of the 80 experimental plots.

207

208 *NUE indexes*

209 We followed indications from previous studies, which measured NUE indexes across
210 different ecosystems (Berendse & Aerts 1987, Bridgham et al. 1995; Pastor & Bridgham
211 1999; Dobermann 2005). Based on these literature studies we identified seven NUE indexes
212 as follows (see Table 1):

213 i) Nitrogen use efficiency (NUE_N), which is an index of plant productivity (i.e. yield)
214 per unit of N acquired (i.e. plant N%).

215 ii) Nitrogen response efficiency (NRE_N), which is an index of plant productivity per unit
216 of available N (i.e. rate of net soil N mineralization).

217 iii) Nitrogen uptake efficiency ($NupE_N$), is the ratio of acquired to available N. This is a
218 measure of how effective the plant community is at acquiring soil available N.

219 iv) Partial factor productivity (PFP_N), is a measure of how much plant biomass is
220 produced per unit of fertiliser N applied. This index includes contributions to yield
221 from endogenous N sources, therefore, this index is a measure of plant productivity

per unit of N from exogenous (i.e. N from artificial fertilization) and endogenous (i.e. N from the soil pool) sources.

v) Agronomic efficiency (AE_N), is a measure of yield in response to the product of N use efficiency from applied sources per unit of N fertiliser applied. This index is the same as PFP_N but subtracts the mean yield of control plots to remove potential growth from endogenous sources of soil N from the equation.

vi) Recovery efficiency (RE_N), is a measure of exogenous N recovered in the plant per unit of fertiliser applied. The endogenous N contribution to yield is excluded by subtracting mean values from control plots.

vii) Physiological efficiency (PE_N), is a measure of yield from exogenous sources per unit of N acquired also from exogenous sources. The endogenous productivity response and N uptake are excluded within this equation by subtracting the mean values of control plots.

$NupE_N$, NRE_N and NUE_N indexes enable us to better understand how effectively plants uptake N under different management treatments and in response to changing soil N availability (see Table 1). However, these indexes do not specifically enable us to account for the additional effects of exogenous N inputs (i.e. N added through artificial fertilization) on plant productivity. We thus used specific indexes (see Dobermann 2005) to estimate the effective use of N fertilizer by plants. These are partial factor productivity of applied N (PFP_N), agronomic efficiency of applied N (AE_N), plant recovery efficiency to applied N (RE_N) and physiological efficiency of applied N (PE_N) (Table 1). The remaining index, partial factor productivity of applied N (PFP_N) measures yield in response to fertiliser application without excluding endogenous sources of N (Table 1). The potential contribution from biological nitrogen fixation to NUE is recognised, however, no specific calculations in

246 relation to this group have been carried out due to the very low percent cover of legumes in
247 Nash's Field (<1%).
248 Multiple factors associated with seasonal changes (i.e. temperature and moisture) can
249 affect plant productivity and N dynamics (Agehara & Warncke 2005; Xu & Zhou 2006;
250 Fridley et al. 2016). Consequently, studies on NUE can have a seasonal component.
251 However, our study analyses data from a long-term grassland that has been 'conditioned' by
252 different nutrient treatments for 22 years. Here we analyse data from the peak of the growing
253 season to provide a snapshot of how NUE might be affected by a different combination of
254 treatments.
255
256 *N loss from soils*
257 Soil N retention efficiency (NRtE) was calculated by subtracting mean soil N pool
258 values (Kg N ha^{-1}) of control plots from soil N pools of N-fertilised plots and then by
259 dividing this value by total N fertiliser applied in 22 years (see Table 2). Soil N loss (SNL)
260 was calculated by subtracting the difference of soil N pools between N-fertilised and control
261 plots from the total amount of N applied over 22 years (Table 2).
262
263 *Data analysis*
264 The Nash's Field experiment is laid out as a split plot design with four randomly located
265 blocks (i.e. larger plots) within which nutrient fertilisation, grazing and liming treatments are
266 nested. In our model, blocks were assigned as random effects and the other treatments
267 (grazing, liming and nutrient application) were assigned as fixed effects. Mixed effects
268 ANOVA was performed to test for treatment effects (i.e. nutrient addition, liming and rabbit
269 grazing) on multiple plant NUE indexes (Table 1) and soil N indexes (Table 2). To construct
270 a final model and confirm model assumptions restricted maximum likelihood (REML) was

used. Significant differences between factor levels were tested using post-hoc Tukey tests. Linear regression models were also used to test for potential covariation and relationships between variables. Data were analysed using version 10.0.0 of the JMP statistical software (SAS Institute Inc.).

Results

NUE in response to nutrient fertilization, grazing and liming

We found that N addition either alone or in combination with other nutrients (i.e. P, K, Mg) significantly reduced NUE_N whereas unfertilized (control) and P-fertilized plant communities had the highest NUE_N ($F_{4,79} = 21.6$, $P < 0.0001$; Fig. 1A). Nitrogen response efficiency (NRE) and Nitrogen uptake efficiency (NupE) data did not significantly change across nutrient treatments but had a tendency to be lower under the N-only treatment (Fig. 1a). All NUE indexes that accounted for additional effects of exogenous N inputs (i.e. PFP_N, AE_N, RE_N and PE_N; Fig 1b) were in general lower under the N-only application but were statistically significant ($P = 0.005$; Fig. 1B) only for RE_N.

We found that NUE_N, NRE_N and $NupE_N$ all significantly decreased under grazing ($F_{1,79} = 9.7$, $P < 0.0001$; $F_{1,79} = 5.82$, $P = 0.01$; $F_{1,77} = 5.65$, $P < 0.01$, respectively; Fig. 2A), whereas PFP_N, AE_N, RE_N and PE_N were not significantly affected by rabbit grazing (Fig. 2B). Liming had positively affected NUE_N, NRE_N and $NupE_N$ ($F_{1,79} = 4.4$, $P = 0.04$; $F_{1,79} = 6.23$, $P = 0.01$; $F_{1,79} = 4.17$, $P = 0.04$, respectively; Fig. 3A). Liming also had a tendency to positively affect PFP, AE, RE and PE as well but these results were not significant (Fig. 3B).

Relationships between NUE indexes and key belowground parameters

We found that NUE_N was significantly negatively related to the rate at which N becomes available in soils (i.e. net soil N mineralization) (Fig. 4A). NRE_N and $NupE_N$ are calculated

using net soil N mineralization values so they cannot be used in this assessment to avoid auto correlations. We found that PFP_N, RE_N, AE_N and PE_N were not related to increases in soil N mineralization rates. Only NUE_N was found positively related to soil pH (Fig. 4B). None of the N uptake and soil availability indexes were significantly related to soil C:N, C:P or N:P ratios, whereas PFP_N, RE_N and AE_N all decreased as soil C:N, C:P and N:P ratios increased ($P < 0.05$ for all analyses). There was no relationship between PE_N and soil C:N, C:P and N:P ratios.

Relationship between NUE indexes, plant species diversity and soil N loss/gains

We found that plant species diversity was significantly positively related to NUE_N ($F_{1,79} = 22.5$, $P < 0.0001$) and negatively related to PFP_N ($F_{1,47} = 7.52$, $P = 0.009$) and RE_N ($F_{1,47} = 5.25$, $P = 0.026$). No significant statistical relationships were found between NUE_N (and each of the other NUE indexes; all analyses $P > 0.05$) and soil N loss or soil N retention efficiency. Among management treatments we found that the 'N-only' treatment was associated with a significant increase in soil N retention efficiency ($P = 0.02$).

Discussion

Our findings show how NUE_N (i.e. plant productivity per unit of N acquired) is significantly higher in plants growing on unfertilised soils compared to grassland communities, which grow on chronically N-fertilised soils. This confirms our first hypothesis and agrees with the common view that plant productivity can be significantly reduced in N-poorer soils but plant biomass production per unit of N acquired can be relatively high (Aerts & Chapin 2000). However, there is also evidence that NUE may not respond to changes in soil N content (Nakamura 2002; Yuan et al. 2005). The other two indexes, which mostly account for endogenous N sources ($NupE_N$, NRE_N) were also higher (although not significantly) in

321 unfertilised soils (Fig. 1A). Instead, our evidence is that NUE_N decreased by a factor of 1.6
322 under the 'N-only' and 'All' (all nutrients applied) treatments and by a factor of 1.7 under the
323 NPMg treatment when compared to the control. These estimates agree with previous findings
324 in peatland sites where NUE was 1.6 times lower with high N return rates than in peatland
325 communities exposed to low nutrient return rates (Bridgham et al. 1995).

326 We found that NUE_N was positively related to plant species diversity, mainly because
327 greater plant species numbers were associated with no N additions. In our study we found
328 that the percent cover of specific plant functional groups (i.e. grasses, legumes, forbs) did not
329 influence nitrogen use efficiency of the plant community. This could be partly because
330 grasses contribute on average >70% of the plant community biomass in our experimental
331 plots. However, we did not specifically measure nitrogen use efficiency of specific functional
332 groups or specific plant species and we suggest that further studies could address how species
333 identity could influence N uptake and use under different management treatments.

334 We also found evidence that limitation of key nutrients such as P or Mg can reduce
335 plant NUE. For example, our results show significantly lower NUE under the N-only
336 treatment especially when measured with the RE_N index (Fig. 1b), which is a measure of
337 plant N uptake from N fertiliser sources only. These results suggest that plant N uptake per
338 unit of N applied is greatly increased when N is added together with other nutrients (i.e. N +
339 PMg). Thus the lack of key limiting non-N nutrients may strongly affect NUE (Elser et al.
340 2010). For example, reduced P availability may restrict plant growth as P is required for
341 photosynthesis, nucleic acid synthesis, respiration and enzyme activation (Vance et al. 2003).
342 Whilst our data suggests that P limitation may reduce NUE, no correlation was found
343 between concentrations of available soil P and NUE.

344 Despite potential effects from limiting nutrients, our data shows no significant
345 differences in NUE between treatments with or without potassium (K) (Fig. 1). This is

346 surprising as K is vital for many plant processes including the activation of over 60 enzymes
347 and the production Adenosine Triphosphate – ATP) (Ujwalaranade-Malvi 2011). It could be
348 that these grassland soils have sufficient K to support N use but further studies should address
349 how N and K interact to support plant growth under multiple management practices.

350 We found that grazing (principally by rabbits) significantly reduced NUE_N, $NupE_N$
351 and NRE_N thus confirming our second hypothesis (Fig. 2A). We suggest that these indexes
352 are lower under grazing partly because of increased soil N inputs from animal faeces and
353 urine and partly because of a reduced need by plants to acquire and store N in their tissues.
354 Our results show that NRE_N (biomass production in response to available N) particularly
355 decreases under grazing and this is possibly due to a strong positive grazing effect on rates of
356 soil N mineralization ($P < 0.0001$) rather than a grazing negative effect on plant yields. These
357 results align with findings from Shan et al. (2011), which show that grazing by sheep can
358 decrease NUE (measured as ANPP per unit of N mineralized); however, overall net effects
359 on NUE will depend on grazing intensity and changes in soil temperature and moisture.
360 Grazing intensity without N inputs has been shown to increase NUE following a grass
361 mowing experiment (Keuter et al. 2013). Here mowing simulated the effects of grazing by
362 removing biomass but did not 'replenish' soil nutrient pools with faecal/urine N returns, thus
363 forcing plants to increase NUE in response to biomass removal. We suggest that NUE would
364 have increased (instead of decreasing) in our experimental grassland if grazing pressure had
365 been greater, this is because there may be higher rates of compensatory regrowth, as
366 identified in the grazing lawns hypothesis (McNaughton, 1984).

367 According to our third hypothesis we found that liming determined an increase in
368 NUE_N, $NupE_N$ and NRE_N indexes (Fig. 3A). These indexes significantly increased in limed
369 grasslands (NRE_N by 68%, $NupE_N$ by 52% and NUE_N by 17%) when compared to unlimed
370 grassland plots. The positive liming effect on these NUE indexes may be explained by

liming-induced increases in soil pH. However, this is only directly supported by a positive correlation between NUE and soil pH (Fig. 4B). The fact that $NupE_N$ also increased in limed soils suggests that acquired N by plants increased more than soil available N.

Whilst we found a positive effect on NUE indexes in response to liming, we suggest that these responses may be site-specific and may not translate to non-acidic soils. This is because NUE may be higher in limed soils when compared to non-limed equivalents due to inherent deficiencies associated with acidic soils. For example, in acidic soils plant growth can be limited by nutrient deficiencies (Marschner 1991) that include an increased loss of N through volatilization, leaching and denitrification (Fageria et al. 2005). There can also be an adverse effect on plant morphology, physiology and biochemical process (Baligar et al. 1997). However, there are mechanisms that can lead to higher NUE in un-limed acid soils. One such mechanism, in response to low pH, is changed physiology and a restriction of N uptake. Here, there may be selective pressures to minimise N loss due to reduced ability by plants to uptake N.

Our fourth hypothesis predicted that NUE indexes would decrease with increases in rates of soil N mineralization. We found this expectation confirmed only for NUE_N whereas changes in other indexes were not related to increases in soil N mineralization. It might be that plants benefit from direct N uptake from artificial inorganic N inputs to soils and thus rely less on N becoming available from the mineralization of soil organic nitrogen pools.

Our final hypothesis was that N losses from soils would be lower where plant NUE is higher. We found, however, no relationship between NUE indexes and total soil N losses or soil N retention efficiency. Several studies show that high NUE can result in lower soil N losses (Silla et al. 2004; de Vries et al. 2006; de Vries et al. 2011) including reductions in soil N leaching (de Vries et al. 2012) and N_2O emissions (Ammann et al. 2009). There might be multiple reasons behind the lack of any relationship between NUE and N loss in our system

including: (a) saturation within some N sinks such as within microbes or plants, or on the exchange sites of soil organic matter and minerals (Templer et al. 2012), (b) high levels of nitrification leading to an excess of nitrate, which is more mobile than other forms of nitrogen (Subbarao et al. 2009), (c) poor synchronicity between N supply and demand (Robertson 1997), (d) low levels of physical protection of nitrates within soil aggregates (Smucker et al. 1995), and (e) overall low fungi to bacteria ratios, which can be indicative of dynamic soil systems and higher N losses (de Vries et al. 2006; de Vries et al. 2011). To improve nitrogen use efficiency and reduce N losses from the system we need to improve synchronicity in N inputs and use and ameliorate soil structure. For example, increases in plant diversity could improve synchronicity in N inputs and use by including species which differ in phenology, root depth and element stoichiometry (Hooper & Vitousek 1998; Fornara & Tilman 2009).

Conclusions

Our findings show how NUE is significantly lower in plants growing on soils receiving either inorganic (synthetic N fertiliser) or organic forms of N (from animal waste) or both. Our evidence is the NUE is higher when the contribution of endogenous sources of N (i.e. soil N mineralisation rates) are included in equations to determine good nutrient management practice. Our findings also show how the practice of agricultural liming can significantly increase NUE and this could be related to soil pH-induced effects on soil N availability and N use by plants. NUE_N (i.e. plant biomass N per unit of N acquired) was the index that showed the most significant change in response to multiple management practices. This suggests that a key plant strategy to respond to low nutrient status is to utilise acquired N more efficiently as opposed to capturing additional resources (i.e. by investing in associations with beneficial symbionts). Based on our findings we suggest that a combination of lower nutrient inputs

(NPKMg) together with liming applications could significantly contribute to increasing NUE across managed grasslands while maintaining grass yields in the long-term.

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Fig. 1. Relationships between NUE indexes and applied inorganic nutrient treatments; (A) Nitrogen Use Efficiency (NUE_N), Nitrogen Uptake Efficiency ($NupE_N$) and Nitrogen Response Efficiency (NRE_N); (B) Partial Factor Productivity for applied N (PFP_N), Agronomic Efficiency for applied N (AE_N), Plant Recovery Efficiency of applied N (RE_N), and Physiological Efficiency of applied N (PE_N). $NupE_N$, NRE_N and NUE_N indexes estimate how effectively plants uptake N in response to changing soil N availability. PFP_N, AE_N, RE_N, PE_N account for additional effects of exogenous N inputs (synthetic N fertilizer). Legend: All = all nutrients (N, P, K, Mg); N, P = N or P alone; NPMg = N, P & Mg together. Error bars show ± SE and represent variation among plots receiving the same nutrient treatment. Different letters indicate a significant difference ($P < 0.05$); NS, not significant.

Fig. 2. Relationships between NUE indexes and grazing (abbreviations and descriptions similar to Fig. 1 and Table 1). Error bars show ± SE and represent variation among plots receiving the same nutrient treatment. Different letters indicate a significant difference ($P < 0.05$); NS, not significant.

Fig. 3. Relationships between NUE indexes and agricultural liming ($CaCO_3$) (abbreviations and descriptions similar to Fig. 1 and Table 1). Error bars show ± SE and represent variation among plots receiving the same nutrient treatment. Different letters indicate a significant difference ($P < 0.05$); NS, not significant.

Fig. 4. Relationships between NUE_N and (A) net soil N mineralisation rates, and (B) soil pH.

601 **Table 1**. Seven indexes of plant NUE that were calculated in the Nash's Field long-term
602 grassland experiment, which enable a comparison of the effects of endogenous (i.e. changing
603 soil N availability) and exogenous (i.e. artificial fertilisation) N inputs on plant N uptake and
604 use across experimental plots, which have been regularly fertilised, grazed and limed over 22
605 years.

Name	Calculation	Explanation	References
Nitrogen Use Efficiency	$NUE_N = Y / U$	Yield (Y) per unit of nutrient taken up (acquired) by the plant (U). U is a measure of N concentration (%) in plant biomass (dry weight). Y and U measure yield and uptake in response to both endogenous nutrients and (exogenous) applied N.	(Berendse & Aerts, 1987; Pastor & Bridgham, 1999)
Nitrogen Response Efficiency	$NRE_N = Y / V$	Yield (Y) per unit of nutrient available in the soil (V).	(Bridgham et al. 1995; Berendse & Aerts 1987; Pastor & Bridgham, 1999)
NitrogenUptake Efficiency	$NupE_N = U / V$	Ratio of acquired N (U) to available N (V).	(Pastor & Bridgham 1999; Yuan et al. 2006)
Partial Factor Productivity	$PFP_N = Y_N/F_N$	Yield in fertilised plots (Y_N) per unit of fertilizer applied (F_N) (kg ha^{-1}).	(Dobermann 2005)
Agronomic Efficiency	$AE_N = (Y_N – Y_0)/F_N$	Yield in fertilised plots (Y_N) minus the mean of biomass yield in control plots (Y_0) per unit of fertilizer applied (F_N) (kg ha^{-1}).	(Dobermann 2005)
Plant recovery efficiency of applied N	$RE_N = (U_N – U_0)/F_N$	Biomass N concentration in fertilised plots (U_N) minus the biomass N concentration in unfertilised controls (U_0) per unit of fertilizer applied (F_N) (kg ha^{-1}).	(Dobermann 2005)
Physiological efficiency of applied N	$PE_N = (Y_N – Y_0)/ (U_N – U_0)$	Yield in fertilised plots (Y_N) minus mean yield from unfertilised control plots (Y_0) divided by yield N concentration in fertilised plots (U_N) minus the yield N concentration in unfertilised controls (U_0).	(Dobermann 2005)

606

607 **Table 2**. Two indexes related to changes in soil N retention efficiency, and total soil N loss
608 over 22 years since the long-term grassland experiment was established in 1991 at Silwood
609 Park, Berkshire, UK.
610

Name	Calculation	Explanation	References
Soil N retention efficiency (g soil N g^{-1} N added)	$NRtE = (S_N - S_0)/F_{N22}$	Soil N pools (kg N ha^{-1}) in fertilised plots (S_N) minus mean soil N pools in the control plots (S_0) divided by total N fertiliser applied (kg N ha^{-1}) during the whole experiment (F_{N22}; 22 years).	(Fornara & Tilman 2012)
Soil N loss (Mg N ha^{-1})	$SNL = (F_N) - (S_N - S_0)$	Soil nutrient loss is measured as the total amount of fertiliser applied in 22 years (F_N) minus the difference between soil N pools of fertilised plots (S_N) and control plots (S_0).	(Fornara et al. 2013)

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Fig. 1

(A) (B)

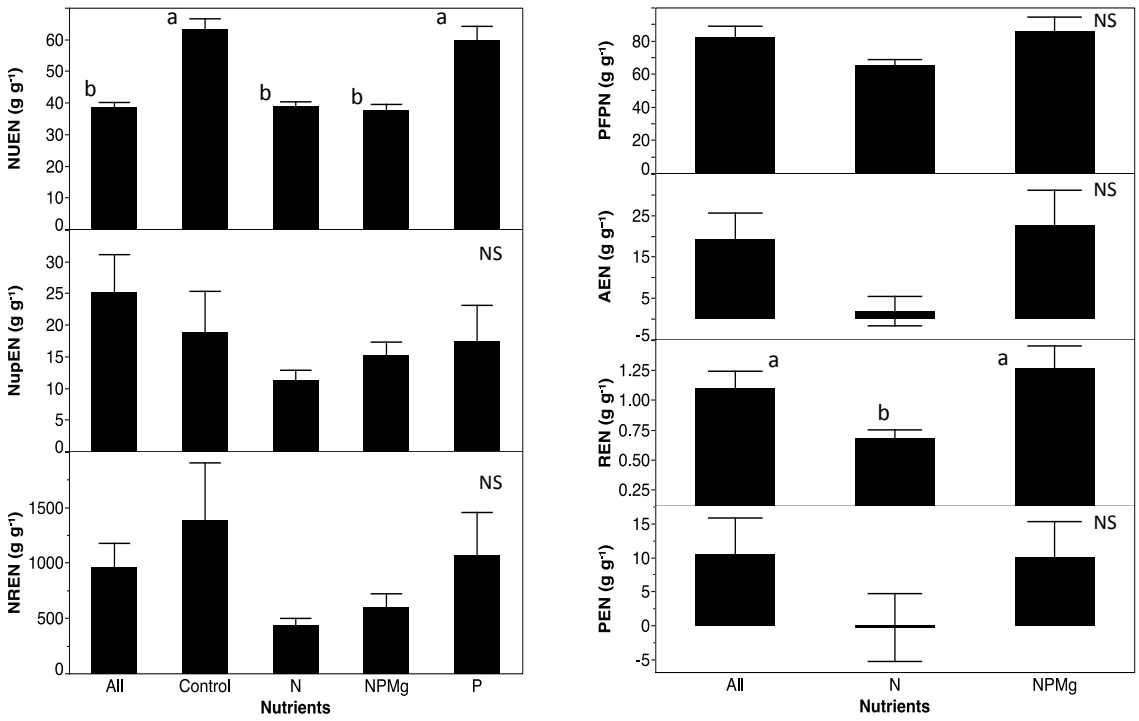


Fig. 2

(A) (B)

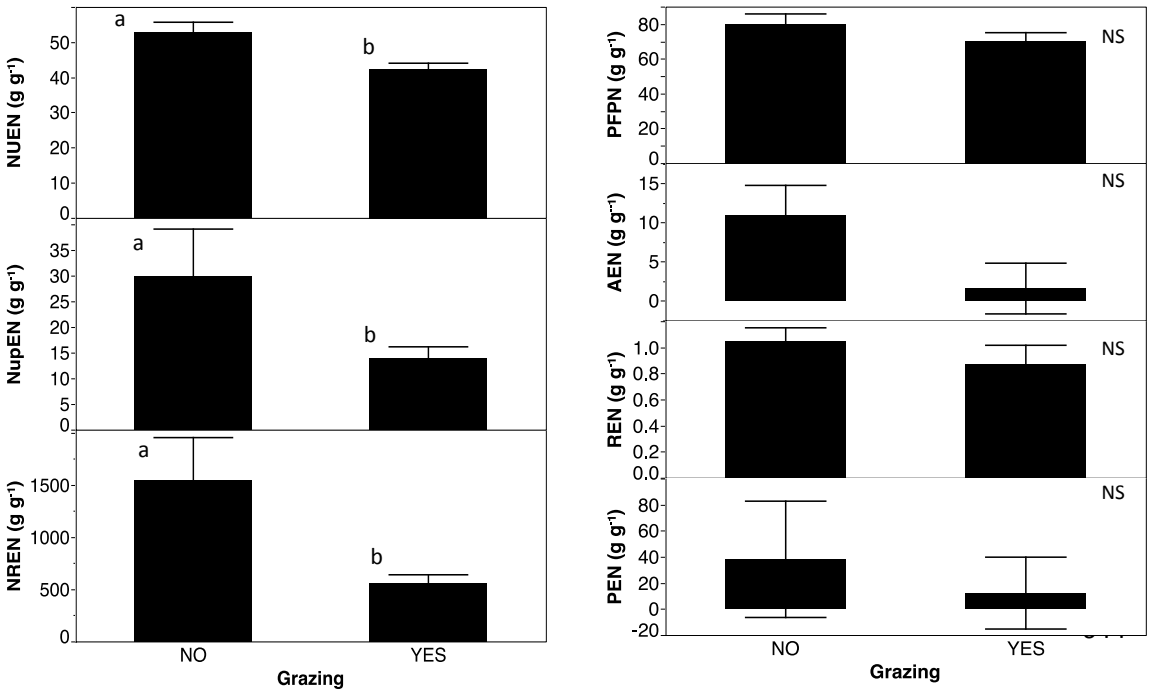


Fig. 3

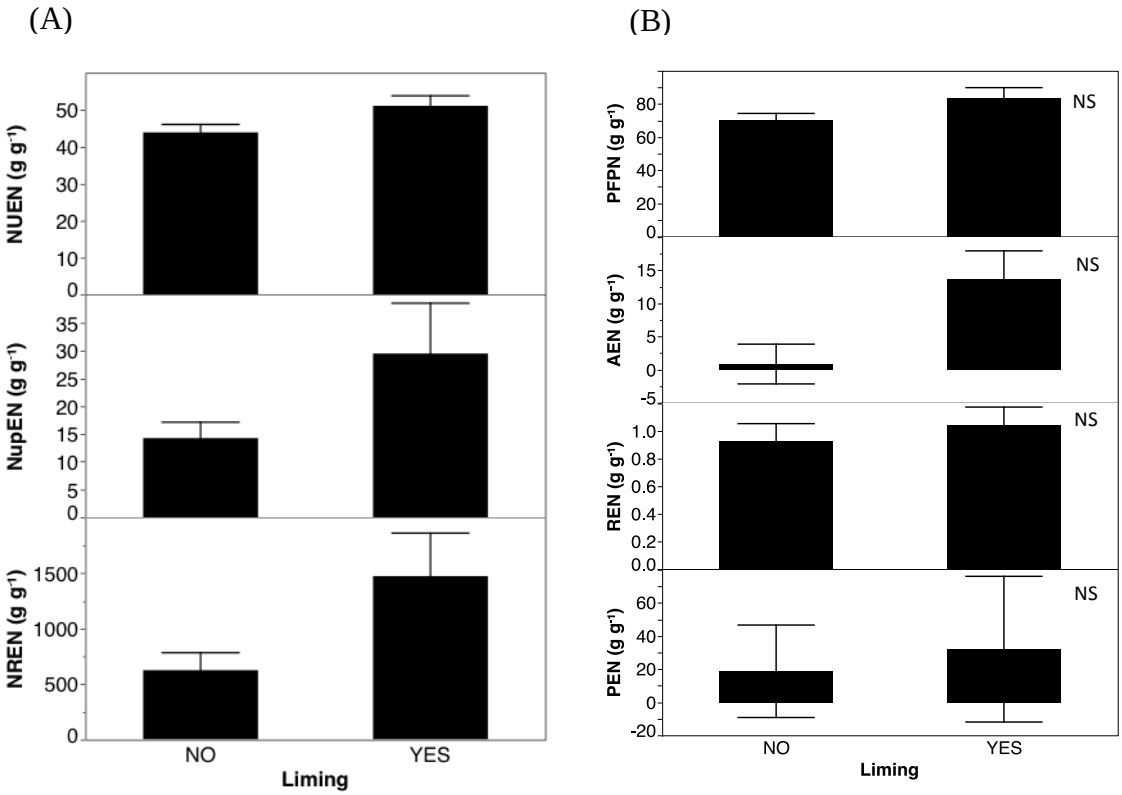
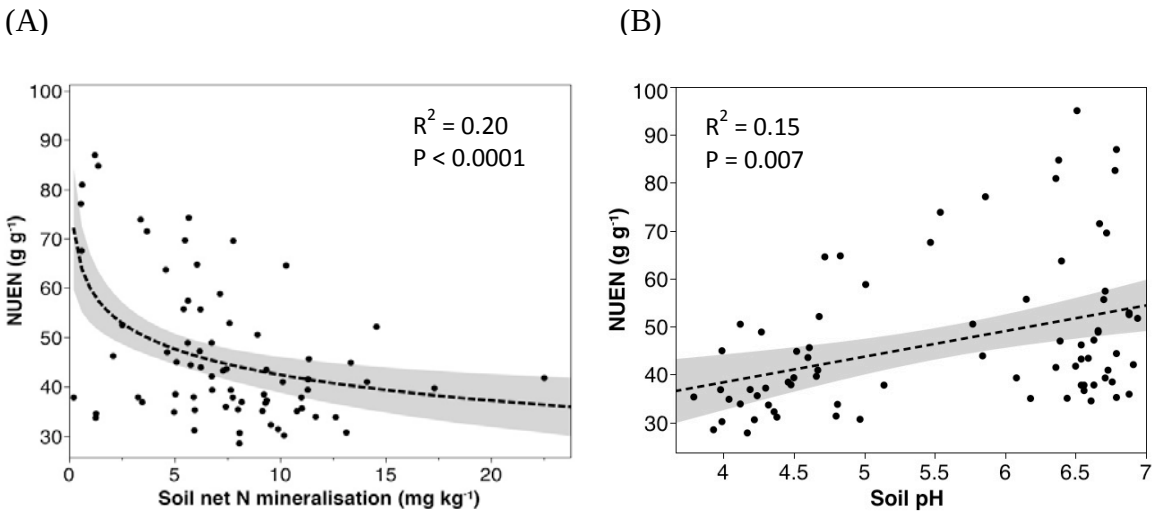

(A)
(B)
NUEN (g g⁻¹)
NupEN (g g⁻¹)
NREN (g g⁻¹)
NO
YES
Liming
PFPN (g g⁻¹)
AEN (g g⁻¹)
REN (g g⁻¹)
PEN (g g⁻¹)
NS
NS
NS
NS
NO
YES
Liming

Fig. 4


(A)
(B)
R² = 0.20
P < 0.0001
NUEN (g g⁻¹)
Soil net N mineralisation (mg kg⁻¹)
R² = 0.15
P = 0.007
NUEN (g g⁻¹)
Soil pH